

Amendments to the Claims:

Claim 1 (Original): A method for generating hydroxylated 14-membered macrolide compounds said method comprising:

- (a) producing a 14-membered aglycone template; and,
- (b) feeding said aglycone template to a strain capable of hydroxylating the aglycone template at the 14 and/or 15 position.

Claim 2 (Original): The method of claim 1, wherein the strain is identified by screening a library of prokaryotes and fungal strains to identify those which are capable of hydroxylating the aglycone template at the 14 and/or 15 position.

Claim 3 (Original): The method of claim 2, wherein the strain is identified by screening a library of actinomycetes.

Claim 4 (Original): The method of claim 1, wherein the strain is selected from the group consisting of *Streptomyces eurythermus*, *Streptomyces avermitilis* and *Streptomyces rochei*.

Claim 5 (Original): The method of claim 1, wherein the strain is selected from the group consisting of *Streptomyces eurythermus* DSM 40014, *Streptomyces avermitilis* ATCC 31272 and *Streptomyces rochei* ATCC 21250.

Claim 6 (Currently Amended): The method of claim 1, wherein the strain used in step (b) is genetically engineered to express a cytochrome P450 capable of hydroxylating the starter unit region of the aglycone template ~~provided said strain~~.

Claim 7 (Original): The method according to claim 6, wherein the recombinant strain used in step (b) is a prokaryote.

Claim 8 (Original): The method according to claim 7, wherein the recombinant strain used in step (b) is *E. coli*.

Claim 9 (Original): The method according to claim 7, wherein the recombinant strain used in step (b) is an actinomycete.

Claim 10 (Original): The method according to claim 9, wherein the recombinant strain used in step (b) is selected from the group consisting of *Saccharopolyspora erythraea*, *Streptomyces coelicolor*, *Streptomyces avermitilis*, *Streptomyces griseofuscus*, *Streptomyces cinnamonensis*, *Streptomyces fradiae*, *Streptomyces eurythermus*, *Streptomyces longisporoflavus*, *Streptomyces hygroscopicus*, *Saccharopolyspora spinosa*, *Micromonospora griseorubida*, *Streptomyces lasaliensis*, *Streptomyces venezuelae*, *Streptomyces antibioticus*, *Streptomyces lividans*, *Streptomyces rimosus*, *Streptomyces albus*, *Amycolatopsis mediterranei*, *Nocardia* sp, *Streptomyces tsukubaensis* and *Actinoplanes* sp. N902-109.

Claim 11 (Currently Amended): The method of ~~any one of claims~~ claim 1 to 10 wherein said hydroxylated 14-membered aglycone product is isolated after step (b).

Claim 12 (Currently Amended): The method of ~~any one of claims~~ claim 1 to 10 which additionally comprises the step of  
(c) feeding the resulting hydroxylated 14-membered aglycone to a second strain which is able to add one or more sugar moieties.

Claim 13 (Original): The method of claim 12 wherein said hydroxylated aglycone produced is fed directly to the strain of step (c) with no purification step.

Claim 14 (Currently Amended): The method of claim 12 ~~or 13~~ wherein the second strain naturally synthesises the desired sugar moiety or moieties and is capable of adding them to the hydroxylated 14-membered aglycone template.

Claim 15 (Currently Amended): The method of claim 12 ~~or 13~~, wherein the second strain is genetically engineered to express and / or transfer the desired sugar moiety or moieties.

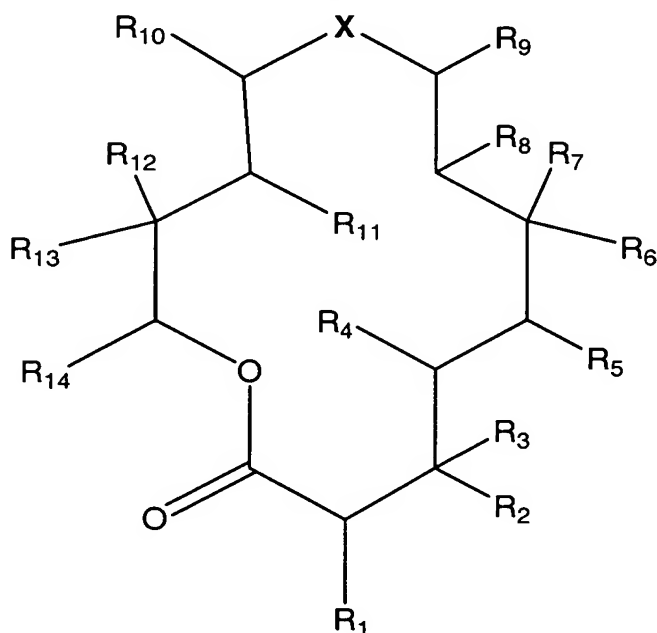
Claim 16 (Original): The method of claim 15, wherein the method of genetically engineering the strain comprises introducing into said strain gene cassette(s) containing the biosynthetic genes

responsible for the synthesis and / or transfer of the desired sugar moiety or moieties.

Claim 17 (Currently Amended): The method according to ~~any one of~~ ~~claims~~ claim 12 to 16, wherein the strain used in step (c) is an actinomycete.

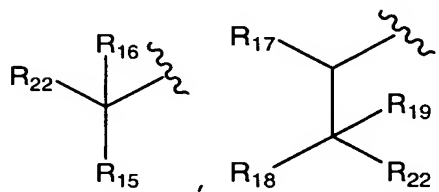
Claim 18 (Original): The method according to claim 17, wherein the strain used in step (c) is selected from the group consisting of *Saccharopolyspora erythraea*, *Streptomyces coelicolor*, *Streptomyces avermitilis*, *Streptomyces griseofuscus*, *Streptomyces cinnamonensis*, *Streptomyces fradiae*, *Streptomyces eurythermus*, *Streptomyces longisporoflavus*, *Streptomyces hygroscopicus*, *Saccharopolyspora spinosa*, *Micromonospora griseorubida*, *Streptomyces lasaliensis*, *Streptomyces venezuelae*, *Streptomyces antibioticus*, *Streptomyces lividans*, *Streptomyces rimosus*, *Streptomyces albus*, *Amycolatopsis mediterranei*, *Nocardia sp.*, *Streptomyces tsukubaensis* and *Actinoplanes sp. N902-109*.

Claim 19 (Currently Amended): The method according to ~~any one of the~~ ~~preceding claims~~ claim 1 wherein the aglycone template fed to said strain in step (b) is according to the formula below:

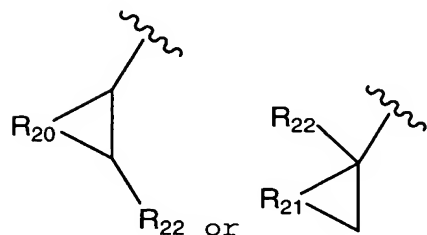


Where:

X = -C(=O)-, -CH(OH)- or -CH<sub>2</sub>-, R<sub>1</sub>, R<sub>4</sub>, R<sub>6</sub>, R<sub>9</sub>, R<sub>10</sub> and R<sub>12</sub> are each independently H, OH, CH<sub>3</sub>, CH<sub>2</sub>CH<sub>3</sub> or OCH<sub>3</sub>; R<sub>2</sub> = OH; R<sub>3</sub> = H; or R<sub>2</sub> and R<sub>3</sub> together are keto; R<sub>5</sub> = OH; R<sub>7</sub> = H, OH or OCH<sub>3</sub>; R<sub>8</sub> = H, OH or keto;



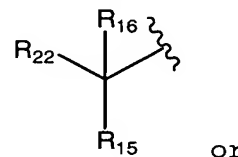
R<sub>11</sub> = H, OH; R<sub>13</sub> = H, OH, and R<sub>14</sub> =



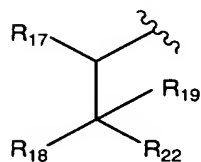
where: R<sub>15</sub> is H or a C<sub>1</sub>-C<sub>7</sub> alkyl group or C<sub>4</sub>-C<sub>7</sub> cycloalkyl group; R<sub>16</sub> is H, a C<sub>1</sub>-C<sub>7</sub> alkyl group or C<sub>4</sub>-C<sub>7</sub> cycloalkyl group, R<sub>17</sub>, R<sub>18</sub> and R<sub>19</sub> are each independently H or a C<sub>1</sub>-C<sub>7</sub> alkyl group or R<sub>20</sub> or R<sub>21</sub> are (CH<sub>2</sub>)<sub>x</sub> where x = 2-5 and R<sub>22</sub> is H; or a variant of a compound as defined above modified by replacing one or more >CHOH or >CHOMe groups by a keto group, or variant of a compound as defined above which differs in the oxidation state of one or more of the ketide units (i.e. selection of alternatives from the group: -CO-, -CH(OH)-, alkene -CH- (=CH- or -CH=), and CH<sub>2</sub>).

Claim 20 (Original): The method of claim 19, wherein

X = -C(=O)-, R<sub>1</sub>=R<sub>4</sub> = R<sub>6</sub> = R<sub>9</sub> = R<sub>10</sub> = R<sub>12</sub> = CH<sub>3</sub>, R<sub>2</sub> = OH, R<sub>7</sub> = H, OH; R<sub>8</sub>



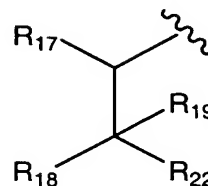
= H, OH, OCH<sub>3</sub>; R<sub>11</sub> = H, OH; R<sub>13</sub> = H, OH; R<sub>14</sub> =



, where: R<sub>15</sub> = H, CH<sub>3</sub>, or CH<sub>2</sub>CH<sub>3</sub> and R<sub>16</sub> is H; or R<sub>17</sub> and R<sub>18</sub> are each independently H or CH<sub>3</sub>; R<sub>19</sub> and R<sub>22</sub> are H.

Claim 21 (Currently Amended): The method of claim 19, wherein:

X = -C(=O)-, R<sub>1</sub>, R<sub>4</sub>, R<sub>6</sub>, R<sub>9</sub>, R<sub>10</sub> and R<sub>12</sub> are each CH<sub>3</sub>, R<sub>2</sub>, R<sub>5</sub> and R<sub>11</sub> =



OH; R<sub>3</sub>, R<sub>8</sub> and R<sub>13</sub> = H; R<sub>7</sub> = H or OH, and R<sub>14</sub> = R<sub>18</sub> where: R<sub>17</sub>, R<sub>18</sub>, R<sub>19</sub> and R<sub>22</sub> = H.

Claim 22 (Original): The method according to claim 6, wherein the oxidative enzyme is identified by screening a library of prokaryotic and fungal strains and cloning the range of oxidative enzymes expressed within a strain capable of hydroxylating the 14-membered aglycone template at the 14 and/or 15 position.

Claim 23 (Original): The method according to claim 22, wherein the library screened is a library of actinomycetes.

Claim 24 (Currently Amended): The method according to claim 22 ~~or claim 23~~, wherein the range of oxidative enzymes within the strain identified as capable of hydroxylating the 14-membered aglycone template at the 14 and/or 15 position are identified using degenerate oligo primers.

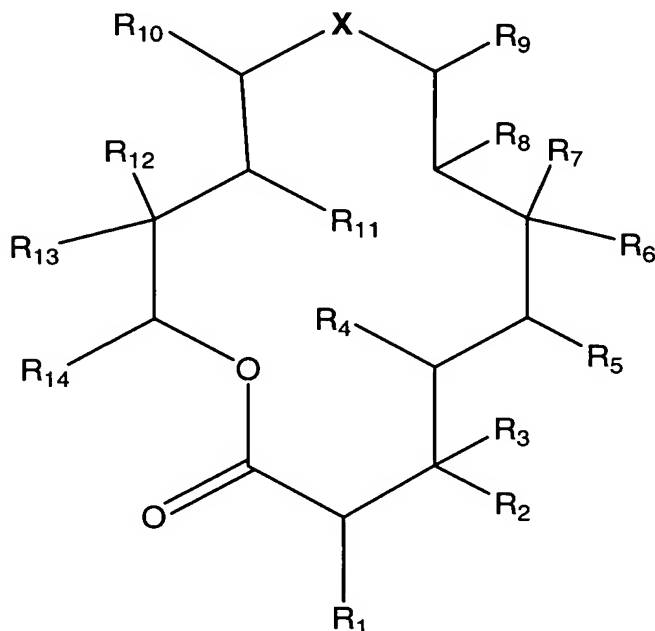
Claim 25 (Currently Amended): The method according to ~~any one of claims~~ claim 22 to 24 wherein the oxidative enzyme(s) is a cytochrome P450.

Claim 26 (Original): A method for generating hydroxylated 14-membered macrolide compounds said method comprising:

- (a) producing a 14-membered aglycone template,
- (b) identifying a cytochrome P450 capable of hydroxylating the 14-membered aglycone template at the 14 and/or 15 position by screening a library of prokaryotic and fungal strains and amplifying the range of P450s expressed within a strain,
- (c) expressing and isolating said P450, and
- (d) using the isolated P450 in vitro to hydroxylate the 14 and/or 15 position of said 14-membered aglycone template.

Claim 27 (Original): The method of claim 26, wherein said P450 is expressed together with appropriate ferredoxin and ferredoxin reductases.

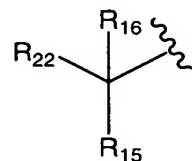
Claim 28 (Currently Amended): A process according to ~~anyone of~~ ~~claims~~ claim 1 to 27 which produces one or more compounds according to formula I:

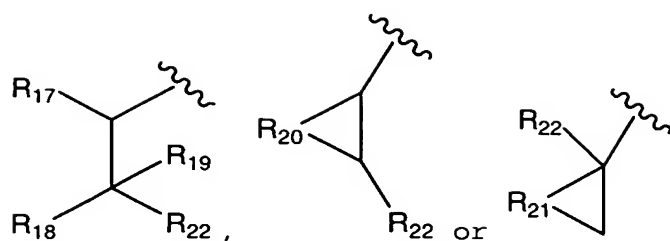


Where:

X = -C(=O)-, -CH(OH)- or -CH<sub>2</sub>-, R<sub>1</sub>, R<sub>4</sub>, R<sub>6</sub>, R<sub>10</sub> and R<sub>12</sub> are each independently H, OH, CH<sub>3</sub>, CH<sub>2</sub>CH<sub>3</sub> or OCH<sub>3</sub>; R<sub>2</sub> = OH, or any glycosyl or disaccharide group, R<sub>3</sub> = H; or R<sub>2</sub> and R<sub>3</sub> together are keto; R<sub>5</sub> = OH, or any glycosyl group, R<sub>7</sub> = H, OH, OCH<sub>3</sub>; R<sub>8</sub> = H, OH or keto; R<sub>9</sub>, = H, OH, CH<sub>3</sub>, CH<sub>2</sub>CH<sub>3</sub> or OCH<sub>3</sub>, O-megosamine, O-cladinose, O-mycarose, O-rhamnose or a methylated derivative thereof, O-digitoxose, O-olivose, O-oliose or O-oleandrose; O-desosamine, O-mycaminose or O-

angolosamine; R<sub>11</sub> = H, OH; R<sub>13</sub> = H, OH, and R<sub>14</sub> =





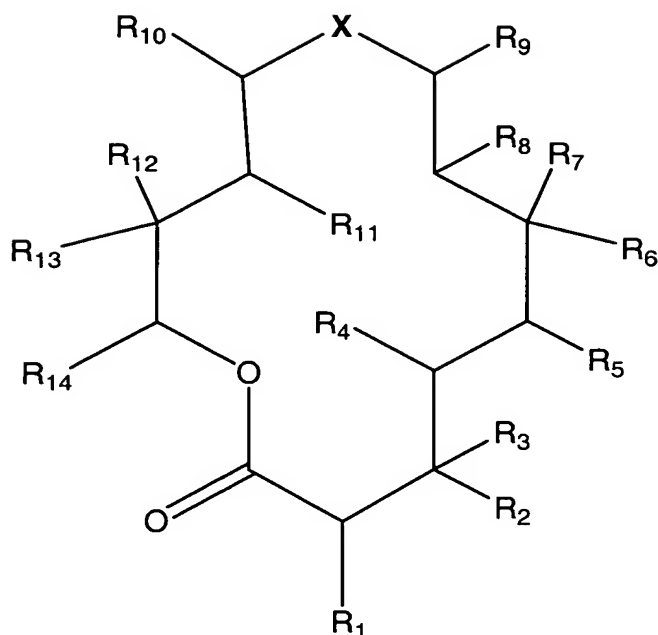
where:  $R_{15}$  is H or a  $C_1$ - $C_7$  alkyl group or  $C_4$ - $C_7$  cycloalkyl group;  $R_{16}$  is H, a  $C_1$ - $C_7$  alkyl group or  $C_4$ - $C_7$  cycloalkyl group,  $R_{17}$ ,  $R_{18}$  and  $R_{19}$  are each independently H or a  $C_1$ - $C_7$  alkyl group or  $R_{20}$  or  $R_{21}$  are  $(CH_2)_x$  where  $x = 2-5$  and  $R_{22}$  is  $O-R_{23}$  where  $R_{23} = H$  or a  $C_1$  to  $C_7$  alkyl group or  $C_1$ - $C_7$  acyl group; or  $R_{22}$  and  $R_{16}$  together are a keto group; or  $R_{22}$  and  $R_{19}$  together are a keto group; or a variant of a compound as defined above which differs in the oxidation state of one or more of the ketide units (i.e. selection of alternatives from the group:  $-CO-$ ,  $-CH(OH)-$ , alkene  $-CH-$  ( $=CH-$  or  $-CH=$ ), and  $CH_2$ ).

Claim 29 (Original): A process according to claim 28 wherein  $R_2$  is selected from *O*-cladinose, *O*-mycarose, *O*-rhamnose and methylated derivatives thereof, *O*-digitoxose, *O*-olivose, *O*-oliose or *O*-oleandrose.

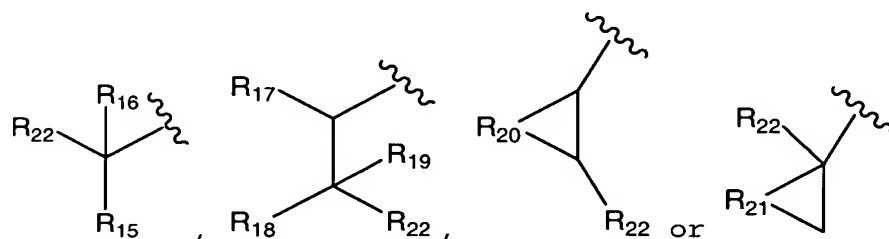
Claim 30 (Original): A process according to claim 29 wherein  $R_2$  and/or  $R_9$  is a said methylated derivative selected from 2'-*O*-methyl, 2',3'-*bis-O*-methyl and 2',3',4'-*tris-O*-methyl.

Claim 31 (Currently Amended): A process according to ~~claims~~ claim 28, ~~29 or 30~~ wherein  $R_5$  is a glycosyl group selected from *O*-mycaminose and *O*-angolosamine.

Claim 32 (Currently Amended): A compound according to formula I below:



wherein X = -C(=O)-, -CH(OH)- or -CH<sub>2</sub>-, R<sub>1</sub>, R<sub>4</sub>, R<sub>6</sub>, R<sub>9</sub>, R<sub>10</sub> and R<sub>12</sub> are each independently H, CH<sub>3</sub> or CH<sub>2</sub>CH<sub>3</sub>, R<sub>2</sub> = OH or any glycosyl group; R<sub>3</sub> = H, or R<sub>2</sub> and R<sub>3</sub> together are keto; R<sub>5</sub> = OH or any glycosyl group; R<sub>7</sub> = H, OH, OCH<sub>3</sub>; R<sub>8</sub> = H, OH; R<sub>11</sub> = H, OH, R<sub>13</sub> = H, OH, R<sub>14</sub> =



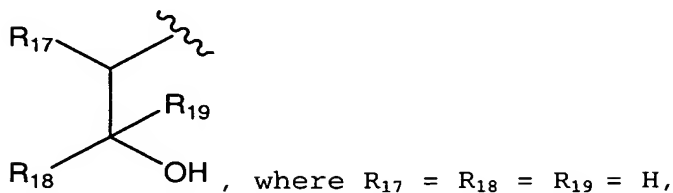
where: R<sub>15</sub> is H or a C<sub>1</sub>-C<sub>7</sub> alkyl group or C<sub>4</sub>-C<sub>7</sub> cycloalkyl group; R<sub>16</sub> is H, a C<sub>1</sub>-C<sub>7</sub> alkyl group or C<sub>4</sub>-C<sub>7</sub> cycloalkyl group, R<sub>17</sub>, R<sub>18</sub> and R<sub>19</sub> are each independently H or a C<sub>1</sub>-C<sub>7</sub> alkyl group or R<sub>20</sub> or R<sub>21</sub> are (CH<sub>2</sub>)<sub>x</sub> where x = 2-5 and R<sub>22</sub> is O-R<sub>23</sub> where R<sub>23</sub> = H or a C<sub>1</sub> to C<sub>7</sub> alkyl group or C<sub>1</sub>-C<sub>7</sub> acyl group; or R<sub>22</sub> = halogen or NR<sub>24</sub>R<sub>25</sub>, where R<sub>24</sub> and R<sub>25</sub> are each independently H, a C<sub>1</sub> to C<sub>7</sub> alkyl group or C<sub>1</sub>-C<sub>7</sub> acyl group; or R<sub>22</sub> and R<sub>16</sub> together are a keto group; or R<sub>22</sub> and R<sub>19</sub> together are a keto group; or a variant of a compound as defined above which differs in the oxidation state of one or more of the ketide units (i.e. selection of alternatives from the group: -CO-, -CH(OH)-, alkene -CH-, and CH<sub>2</sub>); with the proviso that the following compounds are excluded:

(a) when R<sub>2</sub> = OH, O-cladinose or O-mycarose and R<sub>5</sub> is OH or O-



desosamine

(b) when  $R_1 = R_4 = R_6 = R_9 = R_{10} = R_{12} = \text{CH}_3$ ,  $R_3 = \text{H}$ ,  $R_2 = \text{O-oleandrose}$ ,  $R_5 = \text{O-desosamine}$ ,  $R_7 = \text{OH}$ ,  $R_8 = R_{13} = \text{H}$  and  $R_{14} =$



(c) when  $R_2$  or  $R_5 = \text{O-mycaminose}$

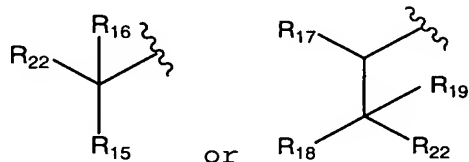
(d) when  $R_2$  or  $R_5 = \text{O-angolosamine}$ .

Claim 33 (Original): A compound according to claim 32 wherein  $R_2$  is selected from *O*-cladinose, *O*-mycarose, *O*-rhamnose and methylated derivatives thereof, *O*-digitoxose, *O*-olivose, *O*-oliose or *O*-oleandrose.

Claim 34 (Original): A compound according to claim 33 wherein  $R_2$  is a said methylated derivative selected from 2'-*O*-methyl, 2',3'-bis-*O*-methyl and 2',3',4'-tris-*O*-methyl.

Claim 35 (Currently Amended): A compound according to claim 32, ~~33~~ ~~or 34~~ wherein  $R_5$  is a glycosyl group selected from *O*-mycaminose and *O*-angolosamine.

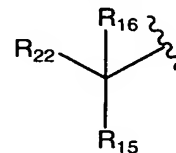
Claim 36 (Currently Amended): A compound according to ~~any of claims 32-35~~ claim 32, where  $X = -\text{C}(=\text{O})-$ ,  $R_1 = R_4 = R_6 = R_9 = R_{10} = R_{12} = \text{CH}_3$ ,  $R_2 = \text{OH}$ , *O*-rhamnose or a methylated derivative thereof, *O*-digitoxose, *O*-olivose, *O*-oliose or *O*-oleandrose,  $R_3 = \text{H}$ ,  $R_5 = \text{OH}$ , *O*-mycaminose or *O*-angolosamine;  $R_7 = \text{H}$ ,  $\text{OH}$ ;  $R_8 = \text{H}$ ,  $\text{OH}$ ,  $\text{OCH}_3$ ;  $R_{11} = \text{H}$ ,



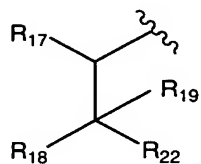
$\text{OH}$ ;  $R_{13} = \text{H}$ ,  $\text{OH}$ ;  $R_{14} = \text{CH}_3$ , or  $\text{CH}_2\text{CH}_3$  and  $R_{16}$  is  $\text{H}$ ; or  $R_{17}$  and  $R_{18}$  are each independently  $\text{H}$  or  $\text{CH}_3$ ;  $R_{19}$  is  $\text{H}$  and  $R_{22}$  is  $\text{OH}$ .

Claim 37 (Original): A compound according to claim 36, where  $X = -\text{C}(=\text{O})-$ ,  $R_1 = R_4 = R_6 = R_9 = R_{10} = R_{12} = \text{CH}_3$ ,  $R_2 = \text{OH}$ , *O*-rhamnose or a

methylated derivative thereof, *O*-digitoxose, *O*-olivose, *O*-oliose or  
*O*-oleandrose;  $R_3 = H$ ;  $R_5 = OH$ , *O*-mycaminose or *O*-angolosamine;  $R_7 =$



$H, OH$ ;  $R_8 = H, OH, OCH_3$ ;  $R_{11} = H, OH$ ;  $R_{13} = H, OH$ ;  $R_{14} =$



, where:  $R_{15} = CH_3$ ;  $R_{16}$  is  $H$ ; or  $R_{17} = R_{18} = R_{19} = H$  and  $R_{22}$   
 is  $OH$ .